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S I R:

Please enter of record in the file of the above application, the attached certified copies of European Patent Application No. 97200047.5 filed on January 9, 1997 and European Patent Application No. 97200830.4 filed on March 19, 1997. The attached European applications are referred to in the Declaration of this application.

Respectfully submitted,

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CERTIFICATE OF MAILING

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Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

97200047.5

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

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Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation

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Application no.: 97200047.5
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Titre de l'invention:
Pet food containing probiotics

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This invention relates to a dried pet food which contains a probiotic micro-organism. In use, the pet food has a beneficial effect in the gastro-intestinal tract of the pet consuming it and hence upon the pet. The invention also relates to a process of producing the pet food and to methods of promoting beneficial effects in the gastro-intestinal tracts of pets.

Probiotic micro-organisms are micro-organisms which beneficially affect a host by improving its intestinal microbial balance (Fuller, R; 1989; J. Applied Bacteriology, 66: 365-378). In general, probiotic micro-organisms produce organic acids such as lactic acid and acetic acid which inhibit the growth of pathogenic bacteria such as *Clostridium perfringens*. Consequently, probiotic bacteria are believed to be useful in the treatment and prevention of conditions caused by pathogenic bacteria. Further, probiotic micro-organisms are believed to inhibit the growth and activity of putrefying bacteria and hence the production of toxic amine compounds. It is also believed that probiotic bacteria activate the immune function of the host.

Therefore there is considerable interest in including probiotic micro-organisms into animal feeds and human foods. For example, Russian patent 2018313 discloses a powdered, spray-dried animal feed which is based upon milk and which contains certain bifidobacteria and streptococci. The animal feed is aimed primarily at live stock although it is mentioned that the feed may be fed to pets.

However, there are two main issues in incorporating probiotic micro-organisms foodstuffs. First, the foodstuff must be in a form which is palatable to the animal or human. Secondly, the probiotic micro-organism must remain viable during storage. The second issue is particularly problematic for dried pet foods which are required to have a storage life of at least a year since the cell counts for many probiotic micro-organisms may fall away completely within one or two days. This is particularly the case if the water activity of the foodstuff is above about 0.5, which is usually the case.

Therefore there is a need for a pet food which contains a probiotic micro-organism, is palatable to pets, and which is storage stable.

Accordingly, in one aspect, this invention provides a dried, cooked pet food which includes a coating or filling which contains a probiotic micro-organism.

It has been found that probiotic micro-organisms remain viable for extended periods of time when formulated into a coating on or filling in a dried, cooked pet food. This is surprising since dried, cooked pet foods generally have a water

activity at levels above about 0.5; levels at which probiotic micro-organisms ordinarily die off rapidly. Therefore the invention offers the advantage of a petfood which is highly palatable to pets and which contains a shelf stable source of probiotic micro-organisms.

Preferably, the pet food comprises a gelatinised matrix of starch and protein; more preferably in the form of pieces or pellets. Preferably the pet food comprises an extrusion cooked pet food.

Preferably the coating comprises a carrier substrate which carries the probiotic micro-organism in it. The filling may also comprise a carrier substrate which carries the probiotic micro-organism in it. For example, the carrier substrate may be fat or a protein digest.

In a further aspect, this invention provides a process of preparing a dried, cooked pet food, the process comprising cooking a starch source and a protein source to form a gelatinised starch and protein matrix; forming the gelatinised matrix into pieces; drying the pieces; and coating the pieces with a coating which contains probiotic micro-organisms.

In a further aspect, this invention provides a process of preparing a dried, cooked pet food, the process comprising cooking a starch source and a protein source to form a gelatinised starch and protein matrix; forming the gelatinised matrix into pieces each containing an aperture; drying the pieces; and filling the aperture of the pieces with a filling which contains probiotic micro-organisms.

Preferably the starch source and protein source are extrusion cooked; extruded through an orifice; and then cut into pieces. The gelatinised starch and protein matrix may be extruded with a central bore for receiving a filling.

Embodiments of the invention are now described, by way of example only, with reference to the drawings in which:

Figure 1 is a graph illustrating the viability of *Bacillus coagulans* in various coatings on a dried, cooked, pet food; and

Figure 2 is a graph illustrating the viability of *Bacillus subtilis* in various coatings on a dried, cooked, pet food.

The invention provides a dried, cooked pet food which includes a coating which contains a probiotic micro-organism. The probiotic micro-organism may be selected from one or more suitable micro-organisms; for example yeasts such as *Saccharomyces*, *Debaromyces*, *Candida*, *Pichia* and *Torulopsis*, moulds such as the genera *Aspergillus*, *Rhizopus*, *Mucor*, and *Penicillium* and bacteria such as the genera *Bifidobacterium*, *Bacteroides*, *Clostridium*, *Fusobacterium*,

Propionibactrium, *Streptococcus*, *Enterococcus*, *Lactococcus*, *Staphylococcus*, *Peptostrepococcus*, *Bacillus*, *Pediococcus*, *micrococcus* and *Lactobacillus*. Specific examples of suitable probiotic micro-organisms are: *Saccharomyces cereviseae*, *Pediococcus acidilactici*, *Bacillus coagulans*, *Bacillus subtilis*, *Bacillus licheniformis*, *Enterococcus faecium*, *Lactobacillus acidophilus*, *Lactobacillus johnsonii*, *Lactobacillus helveticus*, *Lactobacillus sake*, *Lactobacillus delbruckii* subsp. *lactis*, *Lactobacillus farciminus*, *Lactobacillus alimentarius*, *Lactobacillus casei* subsp. *casei*, *Lactobacillus curvatus*, *Lactococcus lactis*, *Streptococcus thermophilus*, *Streptococcus faecalis*, *Pediococcus pentosaceus*, *Pediococcus acidilactici*, *Pediococcus halophilus*, *Staphylococcus xylosus*, *Staphylococcus carnosus*, *Micrococcus varians* and *Bifidobacterium infantis*. The probiotic microorganisms are preferably in powdered, dried form; especially in spore form. Further, if desired, the probiotic microorganism may be encapsulated to further increase the probability of survival; for example in a sugar matrix, fat matrix or polysaccharide matrix.

The dried, cooked pet food may be produced from any suitable ingredients; such as those commonly used in dried, cooked pet foods. Usually these ingredients include a starch source and a protein source. Suitable starch sources are, for example, grains such as corn, rice, wheat, beets, barley, oats, soy, and mixtures of these. Suitable protein sources may be selected from any suitable animal or vegetable protein source; for example meat meal, bone meal, fish meal, soy protein concentrates, milk proteins, gluten, and the like. The choice of the starch and protein sources will be largely determined by the nutritional needs of the animal, palatability considerations, and the type of pet food produced. Various other ingredients, for example, sugar, salt, spices, seasonings, vitamins, minerals, flavouring agents, fats and the like may also be incorporated into the pet food as desired.

The pet food may be produced in many different ways as desired. However, an especially suitable way of producing the pet food is to extrusion cook it. This may be done as is well known in the art. For example, in one suitable process, a feed mixture is fed into a preconditioner. The feed mixture is primarily made up of a starch source and a protein source. If desired, a source of fermentable fibres may also be included; for example chicory, inulin, fructooligosaccharides, soy oligosaccharides and the like. Chicory, which is a source of inulin, is particularly useful. If chicory is included, it preferably comprises at least about 1% by weight of the feed mixture; more preferably at

least about 2% by weight. The maximum level of chicory is preferably about 20% by weight; especially about 10% by weight.

In the preconditioner, water or steam, or both, is mixed into the feed mixture. Sufficient water or steam is mixed into the feed mixture to moisten the feed mixture. If desired, the temperature of the feed mixture may be raised in the preconditioner to about 60°C to about 90°C by weight. A suitable preconditioner is described in US patent 4,752,139. It is not necessary to subject the feed mixture to preconditioning but it is advantageous to do so.

The moistened feed leaving the preconditioner is then fed into an extruder. The extruder may be any suitable single or twin screw, cooking-extruder. Suitable extruders may be obtained from Wenger Manufacturing Inc, Clextral SA, Bühler AG, and the like. During passage through the extruder, the moistened feed passes through a cooking zone, in which it is subjected to mechanical shear and is heated; for example up to a maximum temperature of up to about 150°C, and a forming zone. The gauge pressure in the forming zone is about 300 kPa to about 10 MPa as desired. If desired, water or steam, or both, may be introduced into the cooking zone. During passage through the extruder, the starch source of the moistened feed is gelatinised to provide a gelatinised matrix structure primarily of starch and protein.

The gelatinised matrix leaving the extruder is forced through a suitable die; for example a die as described in European patent application 0665051; the disclosure of which is incorporated by reference. A shaped extrudate, which has a cross-sectional shape corresponding to that of the orifice of the die, leaves the die. If it is desired to produce a centred filled pet food, the gelatinised matrix may be extruded with a central bore. The shaped extrudate is then cut into pieces using blades. The individual pieces are then dried. After drying, the pieces or pellets usually have a water activity of about 0.5 to about 0.7.

The probiotic micro-organisms are then mixed into a suitable carrier substrate. Suitable carrier substrates include animal fats such as tallow, vegetable fats such as hydrogenated soy fat, protein digests which are commonly used as flavour coatings, and water. Suitable protection agents to improve the survival of the micro-organisms may be incorporated into the carrier substrate. Examples of suitable protecting agents are vitamins such as vitamins C and E, amino acids and their salts such as lysine, glycine, cysteine and sodium glutamate, sugars such as lactose, trehalose, saccharose, dextrine and maltodextrine, and proteins such as milk and soya proteins.

The selection of the carrier substrate will depend upon factors such as palatability considerations and the survival of the probiotic micro-organism since some micro-oganisms survive better in some carrier substrates than others. For example, it is found that *S. cereviseae* may be slightly less stable in protein digests than in fats. Also, if fats are used in the carrier substrate, the carrier substrate preferably contains antioxidants to reduce the action of oxygen on sensitive micro-organisms. However selecting the optimum carrier substrate is a matter is simple trial and error for the skilled person. If necessary, the carrier substrate may be heated slightly to melt it or to reduce its viscosity.

For a coated pet food, the mixture of the probiotic micro-organism and the carrier substrate is then sprayed onto the dried pieces or pellets. This may be carried out in any suitable manner. For example, the pieces or pellets may be fed into a fluidized bed onto which the mixture is sprayed. Alternatively, the pieces or pellets may be fed into a rotary coater into which the mixture is sprayed. As a further alternative, the pieces or pellets may be caused to fall in a curtain and the coating mixture sprayed onto the curtain. Also, any technique used for coating the pieces or pellets with flavouring agents may be used.

For a filled pet food, the mixture of the probiotic and micro-organism and carrier substrate is into the central bore of each piece or pellet. In this case, the carrier substrate is preferably viscous or a substance which hardens rapidly. Fats are particularly suitable.

The dried pet food conveniently contains about 10^4 to about 10^{10} cells of the probiotic micro-organism per gram of the dried pet food; preferably about 10^6 to about 10^8 cells of the probiotic micro-organism per gram. The dried pet food may contain about 2% to about 20% by weight of the mixture of the probiotic and micro-organism and carrier substrate; preferably about 3% to about 6% by weight.

If desired, the pieces or pellets may be further coated with protective agents or flavouring agents, or both. This may also be carried out prior to coating or filling the pieces or pellets with the mixture of the probiotic and micro-organism and carrier substrate. The pieces or pellets then may be packed into suitable packages.

As an alternative to producing pieces or pellets, the shaped extrudate leaving the die may be cut into pieces and then formed onto flakes. This may be carried out by feeding the pieces into a suitable flaking apparatus. The flakes are then dried and coated with the coating mixture as described above.

It will be appreciated that the dried, cooked pet food may be produced by any suitable process and not only that described above.

The dried, cooked pet food may be fed to pets as a sole source of nutrition or may be supplemented by other sources of nutrition; for example canned food. Also, the dried, cooked pet food may be fed to the pets in the form of a treat. When consumed in adequate amounts, the dried, cooked pet food results in a production of acids, such as lactic acid and acetic acid, in the gut of the animal. This inhibits the growth of pathogenic bacteria such as *Clostridium perfringens* and has a beneficial effect on the pet. Further, the growth and activity of putrefying bacteria may be inhibited and hence the production of toxic amine compounds. Adequate amounts of the dried, cooked pet food may also result in the activation of the immune function of the pet.

The amount of the dried, cooked pet food to be consumed by the pet to obtain a beneficial effect will depend upon the size of the pet and the type of the pet. However an amount of the dried, cooked pet food to provide a daily amount of about 10^6 to about 10^{12} cells of the probiotic micro-organism would usually be adequate.

Specific examples are now described for further illustration.

Example 1

A feed mixture is made up of corn, about corn gluten, chicken and fish meal, salts, vitamins and minerals. The feed mixture is fed into a preconditioner and moistened. The moistened feed leaving the preconditioner is then fed into an extruder-cooker and gelatinised. The gelatinised matrix leaving the extruder is forced through a die and extruded. The extrudate leaving the die head is cut into pieces suitable for feeding to dogs, dried at about 110°C for about 20 minutes, and cooled to form pellets. The water activity of the pellets is about 0.6.

The pellets sprayed with three different coating mixtures. Each coating mixture contains *Bacillus coagulans* but one coating mixture uses hydrogenated soy fat as a coating substrate, one coating mixture uses water as a coating substrate, and one coating mixture uses a protein digest as a coating substrate. The *B. coagulans* is in the form of powdered endospores and is obtainable from Sankyo Pharmaceutical Company under the trade name Lacris-S. The pellets contain about 1.6×10^6 cells/g of *B. coagulans*. For each coating mixture, the pellets are separated into two groups. One group is stored at about 25°C and, to

obtain an idea of the long term stability of the micro-organism, the other group is stored at about 37°C. A sample is taken of each group is taken after 1 week, 2 weeks, 3 weeks and 4 weeks. Also, a fat coated sample of the group which is stored at 37°C is taken at 8 weeks.

The cell counts are determined for each sample. The results are set out in Figure 1. In all cases, the cell counts remain substantially constant indicating excellent storage stability. Further, the results from the storage at 37°C for 8 weeks indicate that the micro-organisms are likely to be stable after one year of storage at normal conditions.

Example 2

Example 1 is repeated except that the three different coating mixtures each contain *Bacillus subtilis* instead of *Bacillus coagulans*. The *B. subtilis* is in the form of powdered endospores and is obtainable from Hansen A/S under the trade name BioPlus 2B. The results are set out in Figure 2.

In all cases, the cell counts remain substantially constant indicating excellent storage stability. However the cell counts for the pellets coated with fat are a little lower than those for water and protein digest but are still substantially constant. Again, the results from the storage at 37°C for 8 weeks indicate that the micro-organisms are likely to be stable after one year of storage at normal conditions.

Example 3

Example 1 is repeated except that the three different coating mixtures each contain *Pediococcus acidilactici* instead of *Bacillus coagulans*. The *P. acidilactici* is in the form of a dried powder and is obtainable from Lallmand SA under the trade name Bactocell. The storage results are as follows:

Weeks	Fat 25°C	Fat 37°C	Water 25°C	Water 37°C	Digest 25°C	Digest 37°C
0	$19.6x10^6$	$19.6 x10^6$	$21.9 x10^6$	$21.9 x10^6$	$12.9 x10^6$	$12.9 x10^6$
1	$13.6 x10^6$	$13.6 x10^6$	$14.7 x10^6$	$14.7 x10^6$	$12.1 x10^6$	$2.93 x10^6$
2	$12.9 x10^6$	$12.9 x10^6$	$13.1 x10^6$	$13.1 x10^6$	-	-
3	$9.73 x10^6$	$6.69 x10^6$	$16.0 x10^6$	$6.07 x10^6$	$7.77 x10^6$	$0.76 x10^6$
4	$12.9 x10^6$	$4.6 x10^6$	$14.0 x10^6$	$5.31 x10^6$	-	-
5	-	-	-	-	$5.1 x10^6$	$0.68 x10^6$
8	$6.8 x10^6$	$1.5 x10^6$	-	-	-	

For the pellets coated using water or fats, the cell counts remain substantially constant at about 10^7 cfu/g; indicating excellent storage stability. For the pellets coated using protein digest, when stored at 37°C, the cell counts initially fall off but then stabilise at about 10^6 cfu/g; which is adequate.

Example 4

Example 1 is repeated except that the three different coating mixtures each contain *Saccharomyces cereviseae* instead of *Bacillus coagulans*. The *S. cereviseae* is in the form of a dried powder and is obtainable from Santel SA under the trade name Levucell. The storage results are as follows:

Weeks	Fat 25°C	Fat 37°C	Water 25°C	Water 37°C	Digest 25°C	Digest 37°C
0	$28.0 x10^6$	$28.0 x10^6$	$27.6 x10^6$	$27.6 x10^6$	$11.3 x10^6$	$11.3 x10^6$
1	$23.2 x10^6$	$23.3 x10^6$	$17.2 x10^6$	$17.2 x10^6$	$7.53 x10^6$	$1.83 x10^6$
2	$24.5 x10^6$	$24.5 x10^6$	$18.7 x10^6$	$18.7 x10^6$	-	-
3	$24.5 x10^6$	$9.93 x10^6$	$13.5 x10^6$	$4.40 x10^6$	$1.99 x10^6$	$0.16 x10^6$
4	$13.7 x10^6$	$15.9 x10^6$	-	-	-	-
5	-	-	-	-	$2.42 x10^6$	$0.03 x10^6$
8	$17.5 x10^6$	$12.3 x10^6$	-	-	-	

For the pellets coated using water or fats, the cell counts remain substantially constant at about 10^7 cfu/g; indicating excellent storage stability.

This is particularly the case for the pellets coated with fats However the cell counts for the pellets coated with protein digest are a little lower than those for water and fat but are still acceptable when stored at 25°C. When stored at 37°C, the cell counts for the pellets coated with protein digest fall off.

Example 5

A trial is conducted using 30 dogs. The dogs are fed a standard dried diet for a week prior to commencement of the trials. Immediately prior to commencement of the trials, the gut flora and a measure of the faecal odours for each dog is determined.

The dogs are then separated into two groups of 15 dogs. One group of dogs is fed the dried, fat coated pellets of example 1. The other group of dogs is fed the same pellets but without the coating of fat and probiotic micro-organism. The dogs are given free access to the food and to water.

After a week, the gut flora of each dogs is analysed. The dogs which are fed the pellets of example 1 have decreased counts of *C. perfringens*. Further, faecal pH and odours are found to have decreased in the dogs which are fed the pellets of example 1.

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Claims

1. A dried, cooked pet food which includes a coating or filling which contains a probiotic micro-organism.

2. A pet food according to claim 1 which comprises a gelatinised matrix of starch and protein.

3. A pet food according to claim 2 in which the gelatinised matrix is obtained by extrusion cooking.

4. A pet food according to any of claims 1 to 3 in the form of pellets.

5. A pet food according to any of claims 1 to 4 in which the coating or filling comprises a carrier substrate which carries the probiotic micro-organism.

6. A pet food according to claim 5 in which the carrier substrate is a fat or a protein digest.

7. A pet food according to any of claims 1 to 6 which contains about 10^6 to about 10^8 probiotic micro-organisms per gram.

8. A pet food according to any of claims 1 to 7 in which the probiotic micro-organism is selected from *Saccharomyces cereviseae, Pediococcus acidilactici, Bacillus coagulans, Bacillus subtilis, Bacillus licheniformis, Enterococcus faecium,* and *Bifidobacterium* sp., or mixtures thereof.

9. A process of preparing a dried, cooked pet food, the process comprising cooking a starch source and a protein source to form a gelatinised starch and protein matrix; forming the gelatinised matrix into pieces; drying the pieces; and coating the pieces with a coating which contains probiotic micro-organisms.

10. A process according to claim 9 in which the starch source and protein source are extrusion cooked; extruded through an orifice; and then cut into pieces.

Abstract

A dried, cooked pet food which includes a coating which contains a probiotic micro-organism. The pet food may be in the form of pellets. The pet food may be produced by cooking a starch source and a protein source to form a gelatinised starch and protein matrix; forming the gelatinised matrix into pieces; drying the pieces; and coating or filling the pieces with a carrier which contains probiotic micro-organisms.

BMc 7/1/97

Figure 1

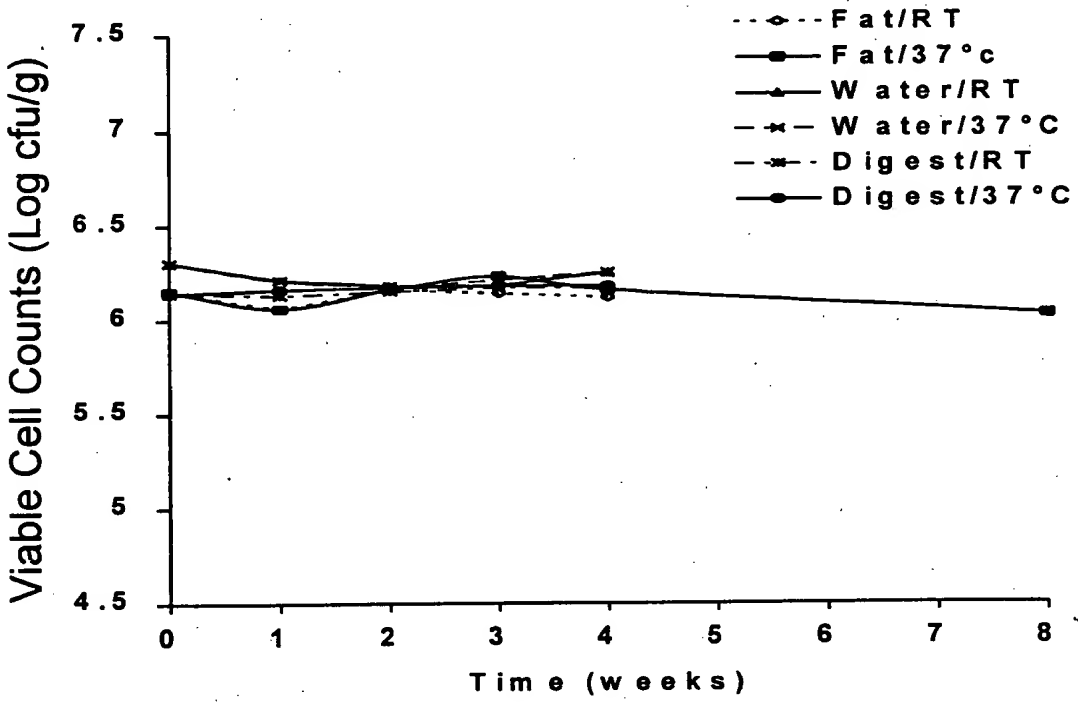

Figure 2